PRODUCT NAME

Product Name: PNAClamp™ Mutation Detection Kit
Brand Name: PNAClamp™ KRAS Mutation Detection Kit (Ver.4)

INTENDED USE

The PNAClamp™ KRAS Mutation Detection Kit (Ver.4) is an in vitro diagnostic test to detect 40 somatic mutations in the KRAS oncogene (Table 1). The kit is to be used by trained laboratory professionals, within a laboratory environment, using (for example) DNA extracted from formalin-fixed paraffin-embedded samples of lung and colorectal biopsies and surgical tissue samples.
The kit is for *in vitro* diagnostic use.

Please read the instructions carefully prior to use.

The PNAClamp™ KRAS Mutation Detection Kit (Ver.4) is a CE marked diagnostic device in accordance with the European Union *in vitro* Diagnostic Medical Device Directive 98/79/EC.
It is MFDS approved for clinical use in Korea.

Table 1. KRAS mutations detected by this kit

No.	Reagent	Exon	Amino Acid Change	Nucleotide change	Cosmic No.
1	G12 PNA mix	2	p.G12S	c.34G>A	517
			p.G12R	c.34G>C	518
			p.G12C	c.34G>T	516
			p.G12D	c.35G>A	521
			p.G12A	c.35G>C	522
			p.G12V	c.35G>T	520
2	G13 PNA mix		p.G13S	c.37G>A	528
			p.G13R	c.37G>C	529
			p.G13C	c.37G>T	527
			p.G13D	c.38G>A	532
			p.G13A	c.38G>C	533
			p.G13V	c.38G>T	534

PRINCIPLE AND OVERVIEW

The PNAClamp™ KRAS Mutation Detection Kit (Ver.4) is based on peptide nucleic acid (PNA)-mediated real-time PCR clamping technology. PNA is a synthetic DNA analog in which the phosphodiester backbone is replaced by a peptide-like repeat formed by (2-aminoethyl)-glycine units.

PNA-mediated real-time PCR clamping relies on the following two unique properties of PNA probes. First, PNA will hybridize to its complementary DNA target sequence only if the sequence is in complete match. Since PNA/DNA duplexes are more thermodynamically stable than the corresponding DNA-DNA duplexes, even with a single mismatch, PNA will not bind to complementary DNA strand, unlike DNA. Second, PNA oligomers are not recognized by DNA polymerases and will not be utilized as primers in subsequence real-time PCR. Instead, it serves as a sequence-selective clamp that prevents amplification during subsequent PCR.

When there is a mutation in target gene and therefore a mismatch is present, the DNA/PNA duplex is destabilized, allowing strand elongation from a bound DNA oligomer which serves as a PCR primer. The outcome is the positive reaction in real-time PCR from the samples harboring mutant allele, while amplification of the wild-type gene is suppressed.

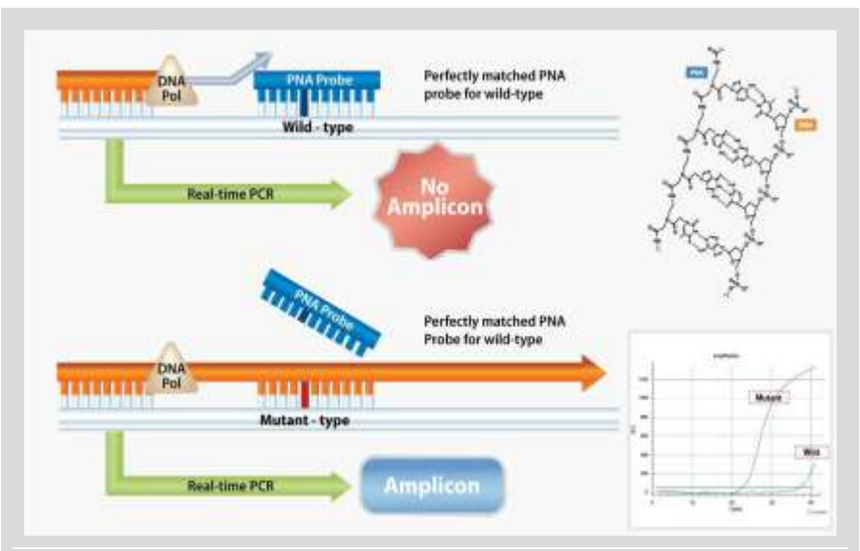


Figure 1. Principle of the PNAClamp™ KRAS Mutation Detection Kit (Ver.4)

The kit can rapidly detect KRAS mutation (within 2 h) with high sensitivity even with a small amount of DNA (10 ng). The detection limit of the kit, when the mutated gene is mixed with wild-type background, is less than 2%.

WARNINGS AND PRECUATIONS

Please read the instruction carefully and become familiar with all components of the kit prior to use.

PNAClamp™ KRAS Mutation Detection Kit(Ver.4) is for *in vitro* diagnostic use.

All experiments should be performed under proper sterile conditions with aseptic techniques. It recommended that users have separate, dedicated pipettes and filter pipette tips to add DNA template and during the preparation of reagents.

Always wear powder-free gloves when you handle the kit.

To avoid repeated freezing and thawing, aliquot all reagents into appropriate volumes and store frozen until use. Thaw appropriate volumes of reagents before each experiment.

All experimental procedures should be performed at room temperature. However, exposing KRAS PNA 2X premix at room temperature should be minimized for the optimal amplification.

Dissolve reagents completely and mix them thoroughly by vortex.

The KRAS PNA 2X premix solution contains fluorescence dye and should be kept dark.

If DNA has been extracted from a paraffin block, additional purification steps may be required.

PCR tubes should be weakly centrifuged before use.

Using non-recommended volume for reagent not only result in loss of performance but also increase the chance of false result.

Using non-recommended volume and concentration for target DNA sample not only result in loss of performance but also increase the change of false result.

Do not exchange and mix up different lots or other manufacture's product.

Upon using instruments, use only recommended consumables only. If not, instruments will not be usable or false result may prominent.

Additional validation testing by user may necessary when using non-recommended instruments.

Do not re-use any remaining reagents after PCR amplification is completed.

Do not use the reagents beyond the expiry date.

STORAGE CONDITION AND STABILITY

The PNAClamp™ KRAS Mutation Detection Kit (Ver.4) is shipped on ice package and must still be frozen on arrival. If the kit is not frozen on arrival please contacts PANAGENE Inc. or the local distributor.

The PNAClamp™ KRAS Mutation Detection Kit (Ver.4) should be stored immediately upon receipt at -15℃ to -20℃. When stored under the recommended storage conditions in the package, the kit is stable until the labeled expiration date.

After opening the kit, shelf-life is 3 months.

KIT CONTENTS

Store at -15℃ to -20℃

No.	Name of component	Description	Volume	Cap label
1	Non PNA mix	Primers only	100 l	KRAS 1
2	G12 PNA mix	G12 PNA and primers	100 l	KRAS 2
3	G13 PNA mix	G13 PNA and primers	100 l	KRAS 3
4	A59 PNA mix	A59 PNA and primers	100 l	KRAS 4
5	Q61 PNA mix	G60, Q61 PNA and primers	100 l	KRAS 5
6	K117 PNA mix	K117 PNA and primers	100 l	KRAS 6
7	A146 PNA mix	A146 PNA and primers	100 l	KRAS 7
8	KRAS PNA 2X premix	PCR reaction premix	1,250 l/vial, 2 vials	**KRAS 2X premix**
9	Clamping control	Wild-type DNA	600 l	**KRAS control**

* Each kit contains enough material to test 25 DNA samples for all mutations.

PROCEDURES

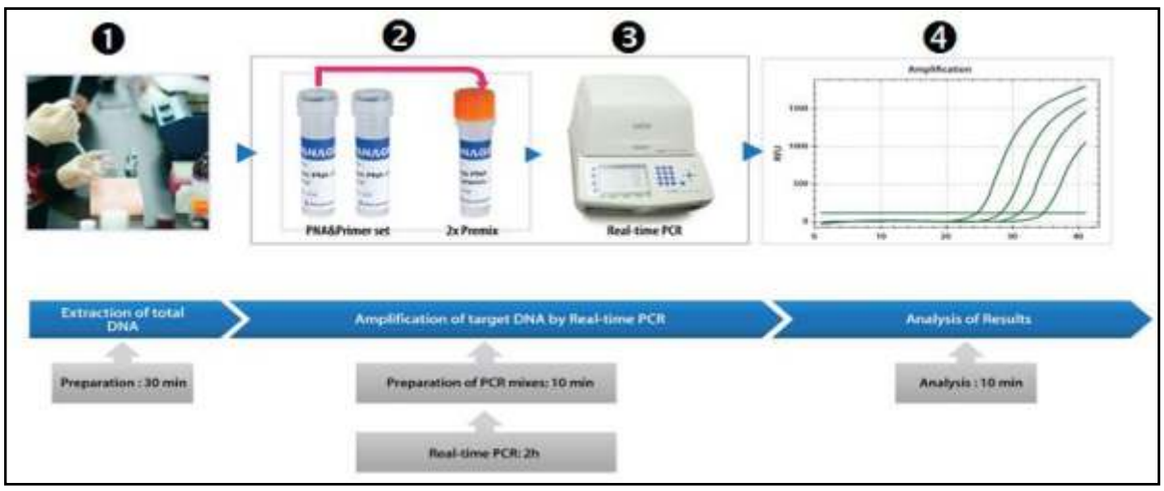


Figure 2. Workflow of the PNAClamp™ KRAS Mutation Detection Kit (Ver.4)

1. DNA preparation

Specimen collection and DNA extraction reagents are not included in the kit so they should be provided by the user.

1) Paraffin embedded tissues or biopsy tissues can be used as specimens.
2) Specimen transport: Use standard pathology methodology to ensure specimen quality.
3) For DNA extraction Kit is recommended below.

Model	Company	Catalog number
PANAMAX™ FFPE DNA Extraction Kit	PANAGENE Inc.	PANK-3001
High Pure PCR Template Preparation Kit	Roche Diagnostics	11796828001
QIAamp DNA FFPE Tissue Kit	Qiagen	56404
QIAamp DNA Mini Kit	Qiagen	51304
Maxwell® 16 FFPE Plus LEV DNA Purification Kit	Promega	AS1135

4) Extracted DNA can be stored at 4℃ for up to 24 hours, or at -20℃ for long term storage.

2. Preparation of the Real-Time PCR Mixture

Table 3. Set up reaction mixture per on reaction.

Components	Volume
KRAS PNA 2X Premix (#8)	10 µl
Each PNA mix (#1~#7)	3 µl
Extracted DNA (10 ng total) or Clamping control (#9)	7 µl
Total volume	20 µl

1) Prepare 7 PCR tubes for one set of DNA samples to be tested. Label them as S1, S2, S3, S4, S5, S6 and S7. Prepare another set of 7 tubes for Clamping control (wild-type DNA) and label them as C1~C7.
2) Add 10 µl of KRAS PNA 2X Premix (#8 from the kit) to each tube.
3) For each PCR tube, add 3 µl of corresponding PNA mix from #1~7 from the kit. For example, S1 and C1 tubes will have #1 Non PNA mix, S2 and C2 tubes will have #2 G12 PNA mix and so forth.
4) For S1~S7 PCR tubes, add 7 µl of prepared DNA sample (10 ng total) to each tube to yield 20 l final volume.
5) For C1~C7 PCR tubes, add 7 µl of Clamping control (#9 from the kit).
6) If you have more than one DNA sample to be tested, prepare one set of Clamping control for the entire experiment. In such case, it is recommended to prepare a master mix containing 2X Premix and each PNA mix for all the samples and to aliquot 13 µl to each PCR tube.
7) When all reagents are loaded, tightly close/seal the PCR tube or 96 well plate. Otherwise, any remaining reagents may evaporate.

3. Real-Time PCR reaction

Perform real-time PCR using the cycling conditions described below

ONE CYCLE		
Pre-denaturation	94℃	5 min
FOUR-STEP CYCLING (40 CYCLES)**		
Denaturation	94℃	30 sec
PNA clamping	70℃	20 sec
Annealing	63℃	30 sec
Extension*	72℃	30 sec

* Set up the detection for reading SYBR Green at 72℃.
** If you use Light Cycler 480 II, Please set up 45 cycles for four-step cycling.

4. Assessment

*** Refer to the specialized instrument user guide by Panagene for detail analysis method.**

1) Clamping control (wild-type DNA control)

(1) Determine Ct value from each PCR reaction. The cycle number at which a signal is detected above background fluorescence is termed as the cycle threshold (Ct).

(2) The Ct values of the Clamping control (tube C1~C7) should fall in the range given in Table 4. The assay should be repeated if the values are not in recommended range.

Table 4. The acceptable Ct ranges of Clamping control

Assay	Acceptable Ct range
① Non PNA mix (C1)	23≤ X ≤ 27

Assay	Acceptable ΔCt-1* range
② G12 PNA mix (C2)	< 2
③ G13 PNA mix (C3)	< 2
④ A59 PNA mix (C4)	< 2
⑤ Q61 PNA mix (C5)	< 2
⑥ K117 PNA mix (C6)	< 2
⑦ A146 PNA mix (C7)	< 2

*ΔCt-1 = [Standard Ct] – [Sample Ct or Clamping control Ct], Standard Ct values given in Table 6 below.

2) DNA samples

(1) Determine Ct values of each sample (S1~S7).

i. Ct value of Non PNA mix (S1) should be 23~34.

ii. Ct value of Non PNA mix (S1) can serve as an internal control to indicate the purity and the concentration of DNA. Thus, the validity of the test can be decided by the Ct value of Non PNA mix (S1) as shown in Table 5.

Table 5. The acceptability of samples

Acceptability	Ct value of Non PNA mix(S1)	Descriptions and recommendations
Optimal	23< Ct <30	The amplification and the amount of DNA sample are optimal.
Acceptable	30≤ Ct <34	The target gene was amplified with low efficiency. For more reliable result, it is suggested that repeat PCR reaction with a higher amount of DNA.
Invalid	Ct ≤ 23	Possibility of false positive is high. Repeat the PCR reaction with a lower amount of DNA.
	34≤ Ct	The amplification was failed. Check DNA amount and purity. New DNA prep might be required.

(2) Calculate the ΔCt-1 values by subtracting the sample Ct values (or Clamping control Ct value) from the Standard Ct values given in Table 6. If the Ct of samples is displayed as NA (not applicable), then set Ct value as 38 for further calculation.

*ΔCt-1 = [Standard Ct] – [Sample Ct (S2, S3, S4, S5, S6, S7) or Clamping control Ct]

Table 6. The value of Standard Ct

Instruments	Standard Ct					
	G12 PNA mix	G13 PNA mix	A59 PNA mix	Q61 PNA mix	K117 PNA mix	A146 PNA mix
Bio-Rad CFX96	36	35	34.5	34	35	34.5
Roche LC480	36	35	34.5	34	35	34.5
ABI 7900	36	35	34	34.5	34.5	34
ABI 7500	36	35	34	34.5	34.5	34
ABI StepOnePlus	36	35	34	34.5	34.5	34
Rotor-Gene Q	36	34.5	34	34.5	34.5	34
QuantStudio 5	36.5	35.5	35	35	35.5	35

(3) Calculate ΔCt-2 [Ct value of sample subtracted by Ct value of Non PNA mix].

**ΔCt-2 = [Sample Ct (S2, S3, S4, S5, S6, S7)] – [Non PNA mix Ct (S1)]

(4) Assess the result for each KRAS PNA mix along with the values of ΔCt-1 and ΔCt-2 as given in Table 7.

Table 7. Assessment of the result

ΔCt-1	ΔCt-2	Assessment
2≤ ΔCt-1	ΔCt-2 ≤9	Mutant
	9< ΔCt-2	Wild
0< ΔCt-1 <2	ΔCt-2 ≤4	Mutant
	4< ΔCt-2	Wild
ΔCt-1 ≤0	All value	Wild

(5) Assess the result along with the result for each KRAS PNA mix as given in Table 8.

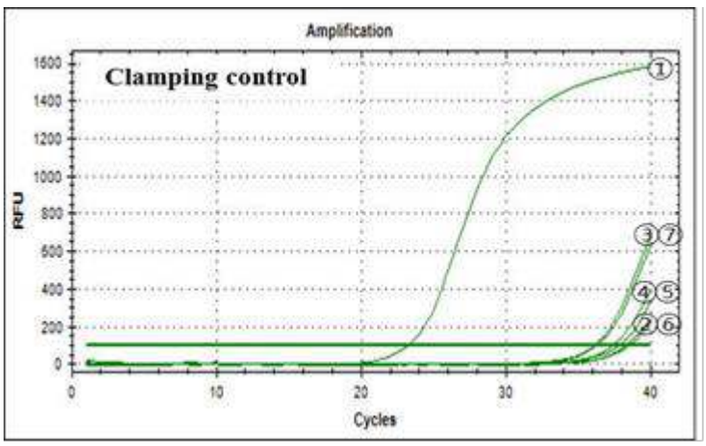
Table 8. Final assessment of the result

G12 PNA mix (S2)	G13 PNA mix(S3)	A59 PNA mix (S4)	Q61 PNA mix (S5)	K117 PNA mix (S6)	A146 PNA mix (S7)	Results
Wild	Wild	Wild	Wild	Wild	Wild	Wild
Mutant	Wild	Wild	Wild	Wild	Wild	Codon 12 mutant
Wild	Mutant	Wild	Wild	Wild	Wild	Codon 13 mutant
Wild	Wild	Mutant	Wild	Wild	Wild	Codon 59 mutant
Wild	Wild	Wild	Mutant	Wild	Wild	Codon 61 mutant
Wild	Wild	Wild	Wild	Mutant	Wild	Codon 117 mutant
Wild	Wild	Wild	Wild	Wild	Mutant	Codon 146 mutant
Mutant	Wild	Wild	Mutant	Wild	Wild	Codon 12 and 61 mutant
Wild	Mutant	Wild	Wild	Wild	Mutant	Codon 13 and 146 mutant

EXAMPLES OF ANALYSIS

1. Using Bio-Rad CFX96

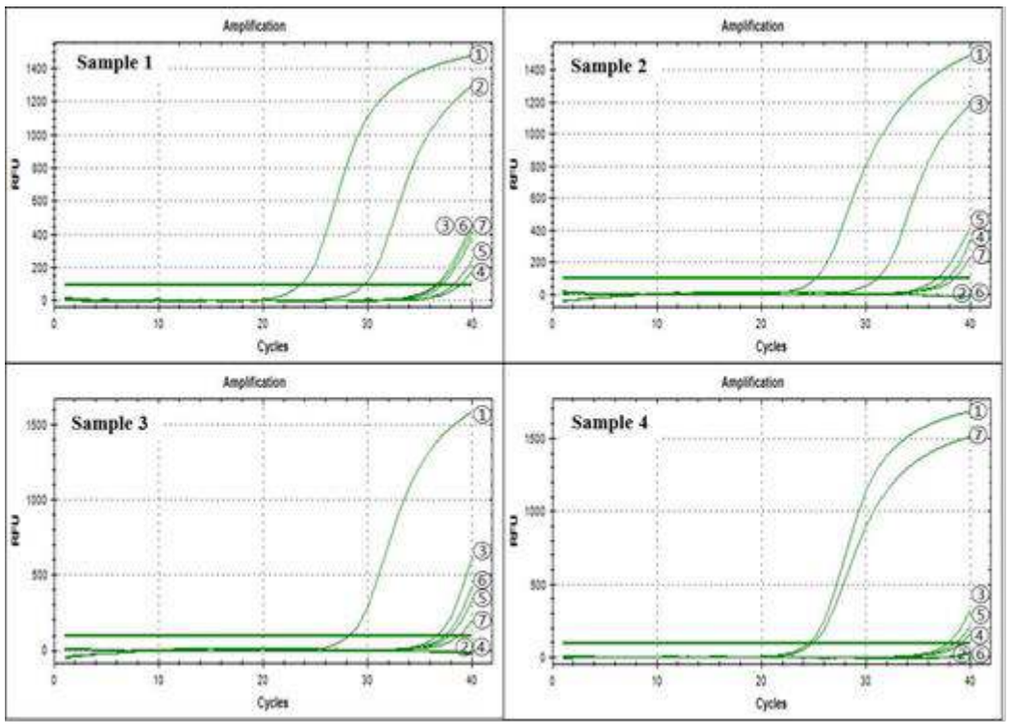
1) Profile of Clamping control



Assay	Clamping control Ct	Accep. range	Result
① Non PNA mix (C1)	23.20	23≤ X ≤ 27	Acceptable

Assay	Clamping control Ct	ΔCt-1	Accep. ΔCt-1 range	Result
② G12 PNA mix (C2)	38.27	-2.27	< 2	Acceptable
③ G13 PNA mix (C3)	36.12	-1.12	< 2	Acceptable
④ A59 PNA mix (C4)	37.34	-2.84	< 2	Acceptable
⑤ Q61 PNA mix (C5)	37.76	-3.76	< 2	Acceptable
⑥ K117 PNA mix (C6)	38.48	-3.48	< 2	Acceptable
⑦ A146 PNA mix (C7)	36.21	-1.71	< 2	Acceptable

2) Profile of samples



① Non PNA mix ② G12 PNA mix ③ G13 PNA mix ④ A59 PNA mix
⑤ Q61 PNA mix ⑥ K117 PNA mix ⑦ A146 PNA mix

Table 9. Example of sample Ct values

Sample No. / Assay	Sample 1 Ct	Sample 2 Ct	Sample 3 Ct	Sample 4 Ct	Standard Ct	**ΔCt-2	*ΔCt-1
① Non PNA mix (S1)	23.62	27.17	28.24	24.51			
② G12 PNA mix (S2)	29.71	38.00	38.00	38.00	36(⑧)	②- ①	⑧- ②
③ G13 PNA mix (S3)	36.62	31.12	36.62	37.92	35(⑨)	③- ①	⑨- ③
④ A59 PNA mix (S4)	38.27	37.76	38.00	39.15	34.5(⑩)	④- ①	⑩- ④
⑤ Q61 PNA mix (S5)	39.01	36.85	37.87	38.55	34(⑪)	⑤- ①	⑪- ⑤
⑥ K117 PNA mix (S6)	36.85	38.00	37.41	38.00	35(⑫)	⑥- ①	⑫- ⑥
⑦ A146 PNA mix (S7)	37.20	38.42	38.77	24.96	34.5(⑬)	⑦- ①	⑬- ⑦

*ΔCt-1 = [Standard Ct] – [Sample Ct or Clamping Control Ct]

**ΔCt-2 = [Sample Ct] – [Non PNA mix Ct (S1)]

Table 10. Analysis of data

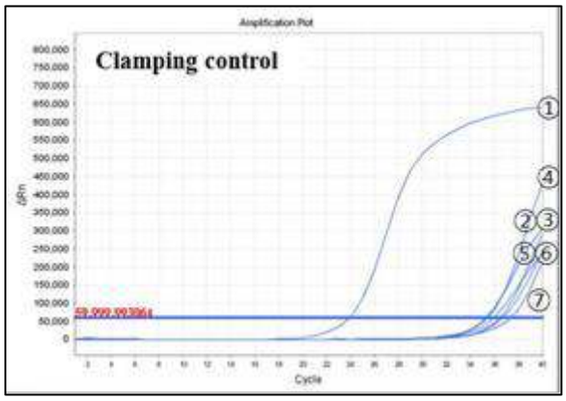
Assay \ Sample No.	Sample 1		Sample 2		Sample 3		Sample 4	
	ΔCt-2	ΔCt-1	ΔCt-2	ΔCt-1	ΔCt-2	ΔCt-1	ΔCt-2	ΔCt-1
② G12 PNA mix (S2)	6.09	6.29	10.83	-2.00	9.76	-2.00	13.49	-2.00
③ G13 PNA mix (S3)	13.00	-1.62	3.95	3.88	8.38	-1.62	13.41	-2.92
④ A59 PNA mix (S4)	14.65	-3.77	10.59	-3.26	9.76	-3.50	14.64	-4.65
⑤ Q61 PNA mix (S5)	15.39	-5.01	9.68	-2.85	9.63	-3.87	14.04	-4.55
⑥ K117 PNA mix (S6)	13.23	-1.85	10.83	-3.00	9.17	-2.41	13.49	-3.00
⑦ A146 PNA mix (S7)	13.58	-2.70	11.25	-3.92	10.53	-4.27	0.45	9.54
Results	Codon 12 mutant		Codon 13 mutant		Wild		Codon 146 mutant	

1. When ΔCt-1 is equal to or greater than 2(▭).
   ① ΔCt-2 is greater than 9, the sample is assessed to be **wild.**
   ② ΔCt-2 is equal to or less than 9(▭), the sample is assessed to be **mutated.**

2. When ΔCt-1 is greater than 0 and less than 2(▭).
   ① ΔCt-2 is greater than 4, the sample is assessed to be **wild.**
   ② ΔCt-2 is equal to or less than 4(▭), the sample is assessed to be **mutated.**

2. Using Roche ABI 7500

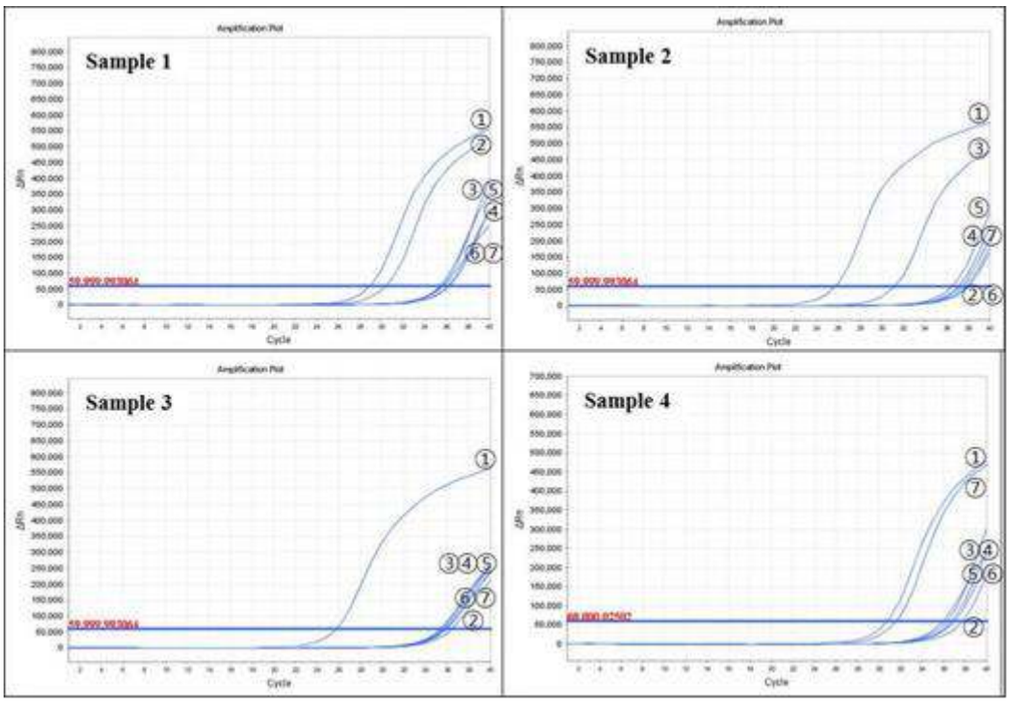
1) Profile of Clamping control



Assay	Clamping control Ct	Accep. range	Result
① Non PNA mix (C1)	23.91	23≤ X ≤ 27	Acceptable

Assay	Clamping control Ct	ΔCt-1	Accep. ΔCt-1 range	Result
① G12 PNA mix (C2)	36.50	-0.50	< 2	Acceptable
② G13 PNA mix (C3)	36.75	-1.75	< 2	Acceptable
③ A59 PNA mix (C4)	35.29	-1.29	< 2	Acceptable
④ Q61 PNA mix (C5)	35.95	-1.45	< 2	Acceptable
⑤ K117 PNA mix (C6)	35.50	-1.00	< 2	Acceptable
⑥ A146 PNA mix (C7)	37.41	-3.41	< 2	Acceptable

2) Profile of samples



① Non PNA mix ② G12 PNA mix ③ G13 PNA mix ④ A59 PNA mix
⑤ Q61 PNA mix ⑥ K117 PNA mix ⑦ A146 PNA mix

Table 11. Example of sample Ct values

Sample No. / Assay	Sample 1 Ct	Sample 2 Ct	Sample 3 Ct	Sample 4 Ct	Standard Ct	**ΔCt-2	*ΔCt-1
① Non PNA mix (S1)	28.98	25.71	25.77	30.79			
② G12 PNA mix (S2)	30.45	38.04	36.42	37.82	36(⑧)	②- ①	⑧- ②
③ G13 PNA mix (S3)	35.39	31.20	35.30	35.95	35(⑨)	③- ①	⑨- ③
④ A59 PNA mix (S4)	35.71	37.14	35.52	36.33	34(⑩)	④- ①	⑩- ④
⑤ Q61 PNA mix (S5)	35.63	36.55	35.71	36.76	34.5(⑪)	⑤- ①	⑪- ⑤
⑥ K117 PNA mix (S6)	36.14	37.82	35.95	36.94	34.5(⑫)	⑥- ①	⑫- ⑥
⑦ A146 PNA mix (S7)	36.31	37.43	35.95	31.59	34(⑬)	⑦- ①	⑬- ⑦

*ΔCt-1 = [Standard Ct] – [Sample Ct or Clamping Control Ct]

**ΔCt 2 = [Sample Ct] [Non PNA mix Ct (S1)]

Table 12. Analysis of data

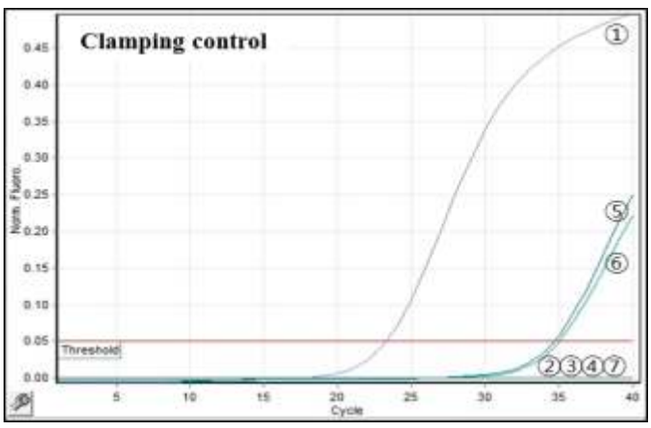
Assay \ Sample No.	Sample 1		Sample 2		Sample 3		Sample 4	
	ΔCt-2	ΔCt-1	ΔCt-2	ΔCt-1	ΔCt-2	ΔCt-1	ΔCt-2	ΔCt-1
② G12 PNA mix (S2)	1.47	5.55	12.33	-2.04	10.65	-0.42	7.03	-1.82
③ G13 PNA mix (S3)	6.41	-0.39	5.49	3.80	9.53	-0.30	5.16	-0.95
④ A59 PNA mix (S4)	6.73	-1.71	11.43	-3.14	9.75	-1.52	5.54	-2.33
⑤ Q61 PNA mix (S5)	6.65	-1.13	10.84	-2.05	9.94	-1.21	5.97	-2.26
⑥ K117 PNA mix (S6)	7.16	-1.64	12.11	-3.32	10.18	-1.45	6.15	-2.44
⑦ A146 PNA mix (S7)	7.33	-2.31	11.72	-3.43	10.18	-1.95	0.80	2.41
Results	Codon 12 mutant		Codon 13 mutant		Wild		Codon 146 mutant	

1. When ΔCt-1 is equal to or greater than 2(▭).
   ① ΔCt-2 is greater than 9, the sample is assessed to be **wild.**
   ② ΔCt-2 is equal to or less than 9(▭), the sample is assessed to be **mutated.**

2. When ΔCt-1 is greater than 0 and less than 2(▭).
   ① ΔCt-2 is greater than 4, the sample is assessed to be **wild.**
   ② ΔCt-2 is equal to or less than 4(▭), the sample is assessed to be **mutated.**

3. Using Rotor-Gene Q

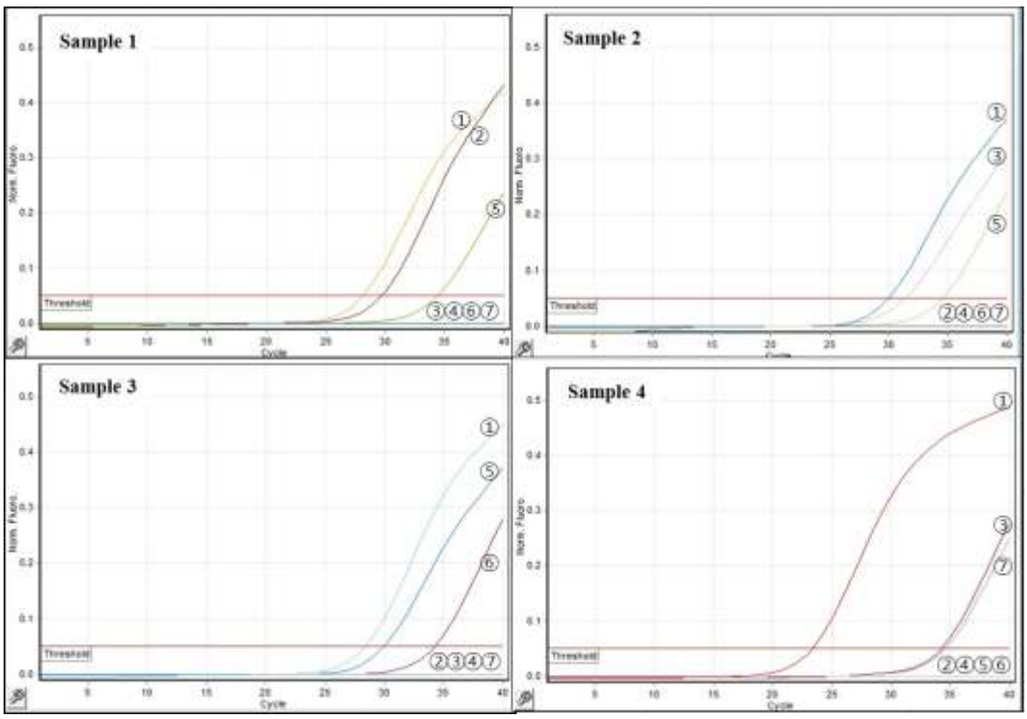
1) Profile of Clamping control



Assay	Clamping control Ct	Accep. range	Result
① Non PNA mix (C1)	23.29	23≤ X ≤ 27	Acceptable

Assay	Clamping control Ct	ΔCt-1	Accep. ΔCt-1 range	Result
① G12 PNA mix (C2)	38.00	-2.00	< 2	Acceptable
② G13 PNA mix (C3)	38.00	-3.50	< 2	Acceptable
③ A59 PNA mix (C4)	38.00	-4.00	< 2	Acceptable
④ Q61 PNA mix (C5)	34.70	-0.20	< 2	Acceptable
⑤ K117 PNA mix (C6)	35.02	-0.52	< 2	Acceptable
⑥ A146 PNA mix (C7)	38.00	-4.00	< 2	Acceptable

2) Profile of samples



① Non PNA mix ② G12 PNA mix ③ G13 PNA mix ④ A59 PNA mix
⑤ Q61 PNA mix ⑥ K117 PNA mix ⑦ A146 PNA mix

Table 13. Example of sample Ct values

Sample No. / Assay	Sample 1 Ct	Sample 2 Ct	Sample 3 Ct	Sample 4 Ct	Standard Ct	**ΔCt-2	*ΔCt-1
① Non PNA mix (S1)	28.12	30.00	28.43	23.35			
② G12 PNA mix (S2)	29.74	38.00	38.00	38.00	36(⑧)	②- ①	⑧- ②
③ G13 PNA mix (S3)	38.00	31.41	38.00	34.29	34.5(⑨)	③- ①	⑨- ③
④ A59 PNA mix (S4)	38.00	38.00	38.00	38.00	34(⑩)	④- ①	⑩- ④
⑤ Q61 PNA mix (S5)	34.54	34.59	30.00	38.00	34.5(⑪)	⑤- ①	⑪- ⑤
⑥ K117 PNA mix (S6)	38.00	38.00	34.62	38.00	34.5(⑫)	⑥- ①	⑫- ⑥
⑦ A146 PNA mix (S7)	38.00	38.00	38.00	34.67	34(⑬)	⑦- ①	⑬- ⑦

*ΔCt-1 = [Standard Ct] – [Sample Ct or Clamping Control Ct]

**ΔCt 2 = [Sample Ct] [Non PNA mix Ct (S1)]

Table 14. Analysis of data

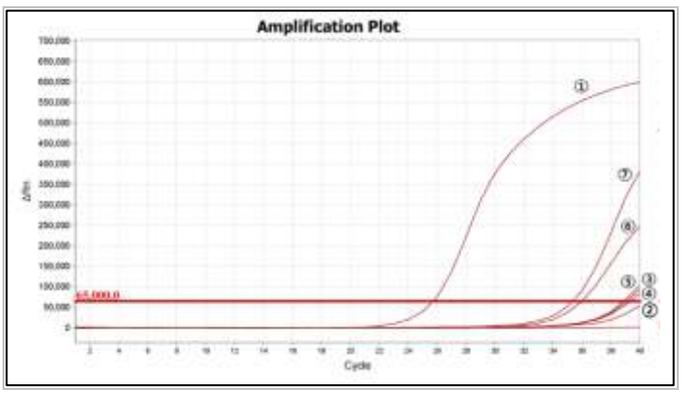
Assay \ Sample No.	Sample 1		Sample 2		Sample 3		Sample 4	
	ΔCt-2	ΔCt-1	ΔCt-2	ΔCt-1	ΔCt-2	ΔCt-1	ΔCt-2	ΔCt-1
② G12 PNA mix (S2)	1.62	6.26	8.00	-2.00	9.57	-2.00	14.65	-2.00
③ G13 PNA mix (S3)	9.88	-3.50	1.41	3.09	9.57	-3.50	10.94	0.21
④ A59 PNA mix (S4)	9.88	-4.00	8.00	-4.00	9.57	-4.00	14.65	-4.00
⑤ Q61 PNA mix (S5)	6.42	-0.04	4.59	-0.09	1.57	4.50	14.65	-3.50
⑥ K117 PNA mix (S6)	9.88	-3.50	8.00	-3.50	6.19	-0.12	14.65	-3.50
⑦ A146 PNA mix (S7)	9.88	-4.00	8.00	-4.00	9.57	-4.00	11.32	-0.67
Results	Codon 12 mutant		Codon 13 mutant		Codon 61 mutant		Wild	

1. When ΔCt-1 is equal to or greater than 2(▭).
   ① ΔCt-2 is greater than 9, the sample is assessed to be **wild.**
   ② ΔCt-2 is equal to or less than 9(▭), the sample is assessed to be **mutated.**

2. When ΔCt-1 is greater than 0 and less than 2(▭).
   ① ΔCt-2 is greater than 4, the sample is assessed to be **wild.**
   ② ΔCt-2 is equal to or less than 4(▭), the sample is assessed to be **mutated.**

4. Using ABI QuantStudio 5

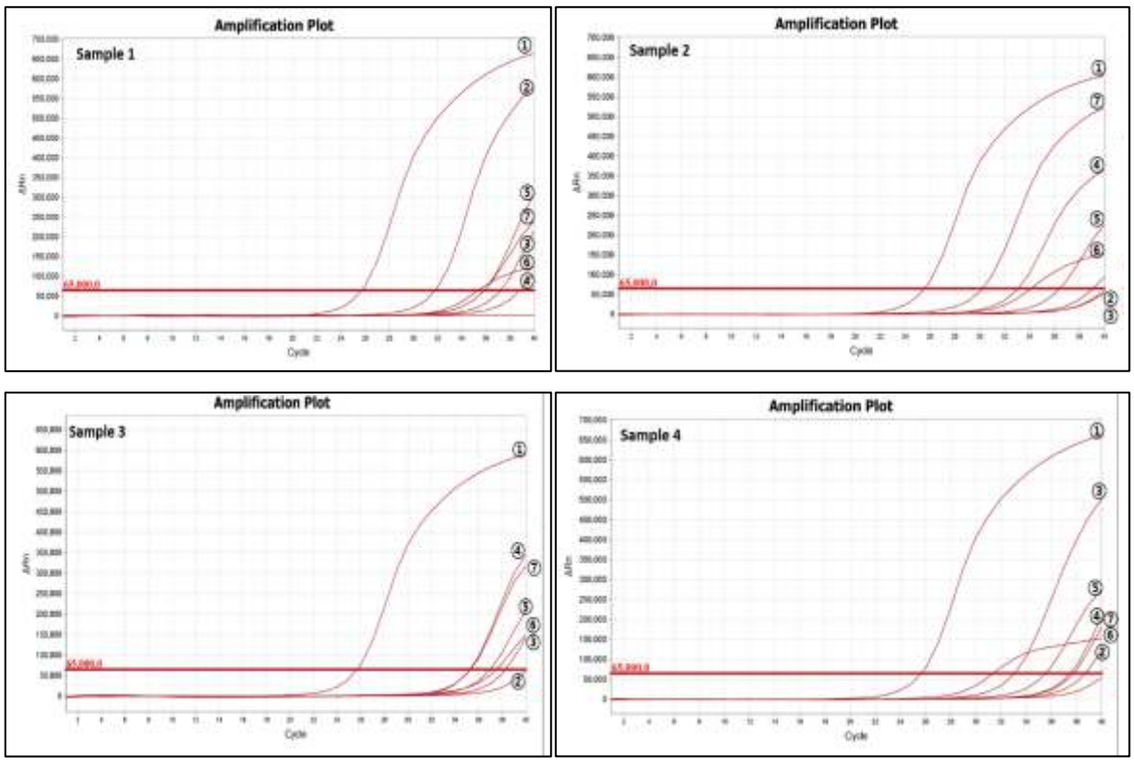
1) Profile of Clamping control



Assay	Clamping control Ct	Accep. range	Result
① Non PNA mix (C1)	25.72	23≤ X ≤ 27	Acceptable

Assay	Clamping control Ct	ΔCt-1	Accep. ΔCt-1 range	Result
② G12 PNA mix (C2)	38.00	-1.50	< 2	Acceptable
③ G13 PNA mix (C3)	38.89	-3.39	< 2	Acceptable
④ A59 PNA mix (C4)	39.09	-4.09	< 2	Acceptable
⑤ Q61 PNA mix (C5)	35.98	-0.98	< 2	Acceptable
⑥ K117 PNA mix (C6)	39.34	-3.84	< 2	Acceptable
⑦ A146 PNA mix (C7)	35.38	-0.38	< 2	Acceptable

2) Profile of samples



① Non PNA mix ② G12 PNA mix ③ G13 PNA mix ④ A59 PNA mix
⑤ Q61 PNA mix ⑥ K117 PNA mix ⑦ A146 PNA mix

Table 15. Example of sample Ct values

Sample No. / Assay	Sample 1 Ct	Sample 2 Ct	Sample 3 Ct	Sample 4 Ct	Standard Ct	**ΔCt-2	*ΔCt-1
① Non PNA mix (S1)	25.72	25.57	25.75	25.68			
② G12 PNA mix (S2)	31.83	38.00	38.00	38.65	36.5(⑧)	②- ①	⑧- ②
③ G13 PNA mix (S3)	37.18	38.00	38.36	33.05	35.5(⑨)	③- ①	⑨- ③
④ A59 PNA mix (S4)	38.80	33.03	35.21	37.47	35(⑩)	④- ①	⑩- ④
⑤ Q61 PNA mix (S5)	35.87	36.41	36.27	35.52	35(⑪)	⑤- ①	⑪- ⑤
⑥ K117 PNA mix (S6)	35.40	34.13	37.37	31.37	35.5(⑫)	⑥- ①	⑫- ⑥
⑦ A146 PNA mix (S7)	35.87	30.21	35.44	37.73	35(⑬)	⑦- ①	⑬- ⑦

*ΔCt-1 = [Standard Ct] – [Sample Ct or Clamping Control Ct]

**ΔCt-2 = [Sample Ct] – [Non PNA mix Ct (S1)]

Table 16. Analysis of data

Sample No. / Assay	Sample 1		Sample 2		Sample 3		Sample 4	
	ΔCt-2	ΔCt-1	ΔCt-2	ΔCt-1	ΔCt-2	ΔCt-1	ΔCt-2	ΔCt-1
② G12 PNA mix (S2)	6.11	4.67	12.25	-1.50	11.98	-1.32	12.97	-2.15
③ G13 PNA mix (S3)	11.46	-1.68	12.61	-2.86	11.30	-1.64	7.37	2.45
④ A59 PNA mix (S4)	13.08	-3.80	9.46	-0.21	12.16	-3.00	11.79	-2.47
⑤ Q61 PNA mix (S5)	10.15	-0.87	10.52	-1.27	10.72	-1.56	9.84	-0.52
⑥ K117 PNA mix (S6)	9.68	0.10	11.62	-1.87	10.83	-1.20	5.69	4.13
⑦ A146 PNA mix (S7)	10.15	-0.87	9.69	-0.44	5.05	4.11	12.05	-2.73
Results	Codon 12 mutant		Wild		Codon 146 mutant		Codon 13, 117 mutant	

1. When ΔCt-1 is equal to or greater than 2(▭).
   - ① ΔCt-2 is greater than 9, the sample is assessed to be **wild.**
   - ② ΔCt-2 is equal to or less than 9(▭), the sample is assessed to be **mutated.**

2. When ΔCt-1 is greater than 0 and less than 2(▭).
   - ① ΔCt-2 is greater than 4, the sample is assessed to be **wild.**
   - ② ΔCt-2 is equal to or less than 4(▭), the sample is assessed to be **mutated.**

QUALITY CONTROL

Each lot of **PNAClamp™ KRAS Mutation Detection Kit (Ver.4)** is tested against predetermined specifications to ensure consistent product quality in accordance with PANAGENE's ISO 9001 & 13485-Certified Quality Management System.

PERFORMANCE TEST

1. Analytical Sensitivity

The analytical sensitivity was determined by testing the standard KRAS mutant samples with the PNAClamp™ KRAS Mutation Detection Kit (Ver.4). The extracted DNA is measured as 10 ng.
The samples were diluted to have 5%, 2% and 1% of the different mutant ratio.
Three tests were performed with these 3 conditions of DNAs for 3 different batches of the kit.
The results showed that 2% mutation was detected for 10 ng DNAs.
Furthermore, the G12, G13, A59, K117 and A146 PNA mix were detected 1% mutation.

2. Analytical Specificity

The analytical specificity was determined by testing the wild cell lines without mutant DNA. Three experiments were performed on three batches of the kit using DNA (10 ng) extracted from wild-type cell line HeLa. All the three tests showed wild-type locations. For the evaluation of the cross-reactivity by mutant location, the tests of six types of mutant DNAs (10 ng) showed wild-type locations, except for each mutant location, and did not show cross-reactivity.

3. Reproducibility

Experiments were performed to evaluate the reproducibility of six standard DNAs (10 ng) at 100, 5, 2, 1 and 0% of different mutant ratio, for three batches, among three operators, and for three days. PNAClamp™ KRAS Mutation Detection Kit (Ver.4) had a correct call rate of 100%. All the results showed little variation, with % CV<5%.

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EXPLANATION OF SYMBOLS ON THE LABEL

IVD	*In Vitro* Diagnostic Medical Device		Manufacturer
LOT	Batch code	Σ	Contains Sufficient for <*n*> tests
REF	Catalogue number		Upper limit of storage temperature
EC REP	Authorized European representative		Use by
	Consult instructions for use	CE	This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.

CE

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